Advantage of animal models with metabolic flexibility for space research beyond Low Earth Orbit

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Abstract: As the world's space agencies and commercial entities continue to expand beyond Low Earth Orbit (LEO), novel approaches to carry out biomedical experiments with animals are required to address the challenge of adaptation to space flight and new planetary environments. The extended time and distance of space travel along with reduced involvement of Earth-based mission support increases the cumulative impact of the risks encountered in space. To respond to these challenges, it becomes increasingly important to develop the capability to manage an organism's self-regulatory control system, which would enable survival in extraterrestrial environments. To significantly reduce the risk to animals on future long duration space missions, we propose the use of metabolically flexible animal models as "pathfinders," which are capable of tolerating the environmental extremes exhibited in spaceflight, including altered gravity, exposure to space radiation, chemically reactive planetary environments and temperature extremes.

In this report we survey several of the pivotal metabolic flexibility studies and discuss the importance of utilizing animal models with metabolic flexibility with particular attention given to the ability to suppress the organism's metabolism in spaceflight experiments beyond LEO. The presented analysis demonstrates the adjuvant benefits of these factors to minimize damage caused by exposure to spaceflight and extreme planetary environments. Examples of microorganisms and animal models with dormancy capabilities suitable for space research are considered in the context of their survivability under hostile or deadly environments outside of Earth. Potential steps toward implementation of metabolic control technology in spaceflight architecture and its benefits for animal experiments and manned space exploration missions are discussed.

Key words: spaceflight, animal research, metabolic flexibility

Introduction: While the increased human presence in orbit over the last four decades has shown that humans can adapt to short duration spaceflight, we still have an incomplete understanding of the adaptation to long-duration spaceflight, and little is known about health related consequences of long-term exposure to the spaceflight environment. Future missions to the Moon, Mars, and other deep space objects such as asteroids or moons of other planets provide extraordinary scientific opportunities for space biologists to explore life's ability to adapt to the spaceflight environment during long duration missions. By studying experimental animals aboard deep space missions, scientists can better understand the adaptive response of life to long duration spaceflight. Results of these missions can help to define the requirements for optimal human health in deep space as well.

Since the of era of orbital flights, animals have always preceded humans in space missions to act as "pathfinders," to help scientists produce new medical knowledge and test engineering design concepts that are required to support human space exploration. Animal models are also recognized as cost-effective solutions to probe fundamental biology questions related to human health. Before Apollo missions to the moon, animals were used only once in space missions beyond Low Earth Orbit (LEO). In 1968, a pair of Russian tortoises with a number of other biological specimens, launched on a trans-lunar mission aboard the Zond 5 spacecraft, and were the first animals from Earth which passed within 1950 km of the lunar surface and returned

safely to Earth.[1] This spacecraft was planned as a precursor to a manned lunar spacecraft. The next time animals were sent beyond LEO was aboard the Apollo 17 mission, launched on December 17, 1972 with the main objective to gain a better understanding of cosmic particle radiation on animal tissues. In this mission most of the pocket mice chosen for the flight experiments successfully survived 13-day journey after orbiting the moon[2].

As long-term exploration missions by Space Agencies and commercial entities continues to evolve and expand, we must design novel approaches to carrying out biomedical experiments with animals based on the necessity of their long presence in space. The payoff will be significant improvement in selecting and designing methods for optimizing adaptation to long duration space flight.

A historical review of all animal experiments in spaceflight research since their first use in the 1950s shows that mortality of animals due to unpredictable failures in the life support systems remains the main issue of concern being higher than the expected 5% level. Although mortality risk among animals in space is significantly minimized in the currently available animal habitat hardware systems developed by the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) to support animal research on International Space Station (ISS), mortality rates still remain high, especially in case of non-manned autonomous missions.[3,4]. Since in extended duration space flights experiments beyond LEO will likely contribute more complications to animal welfare, it becomes important to design novel approaches to ensure animal safety and mission outcome. One approach may be to take advantage of altering an organism's self-regulatory control system, which would allow the animal survive in extraterrestrial environments.

In this report we will briefly survey some of the pivotal studies and discuss the importance of utilizing animal models with metabolic flexibility and particularly the ability to suppress their metabolism in space flight experiments beyond LEO. The presented analysis demonstrates the adjuvant benefits of this selection factor to minimize damage caused by exposure to spaceflight and extreme planetary environments including altered gravity, elevated radiation, chemically reactive planetary dusts, and temperature extremes.

The information, concepts, and hypotheses summarized here warrant additional studies of the new metabolic control strategy with potential to be incorporated in space flight architecture beyond LEO. It is also important to note this strategy is not limited to the category of animals with naturally occurring metabolic flexibility such as hibernating animals. With the current state of biotechnology, many other species, which are not normally capable of inducing metabolic depression, could be intentionally pre-conditioned to this state using the metabolic control technology. The possibility of manipulating metabolic mechanisms in animals and testing it in long-duration missions beyond LEO may lead to development of biomedical technologies capable of sustaining and protecting astronauts from the extreme dangers of the spaceflight environment.

Metabolic Control as a strategy for transportation of experimental animals beyond LEO: With growing interest in spaceflight beyond LEO, it becomes important to ensure the health and physiological performance of animal models under spaceflight environment conditions. Upmass

and power constraints are of paramount importance in considering the logistics of transporting experimental animals into space. In lunar or planetary animal exploration, current animal life support technologies have large space and mass requirements, dramatically increasing cost. In the context of the emerging space and planetary architecture, these constraints are expected to continue, and will greatly limit opportunities for NASA to develop a portfolio of deep space biological research that can answer key questions of interest to both the Exploration Systems Mission Directorate and the Science Mission Directorate. One approach to eliminate expensive life support technologies for nominal animal metabolism during long duration spaceflight would be to apply principals of *metabolic control* upon model organisms. By altering the metabolism of animals to a minimal level during a spaceflight mission, life support requirements are reduced until normal metabolism rates are desired. The phenomenon of the metabolic flexibility, or the ability to reversibly alter metabolism in response to availability and need for energy, is well known for many simple and unicellular organisms. [5,6,7,8]

Metabolic suppression is defined as a drop in standard metabolic rates to less than the normal value with energy-saving benefits toward survival in response to life-threatening environmental stressors. The molecular mechanisms that regulate reversible transitions to and from hypometabolic states are conserved among biologically diverse organisms and include the coordinated reduction of specific cell processes at the genetic, molecular, cellular, organ, and organismal levels. [9,10,11]

Metabolic flexibility in its different forms, extent, and duration, is also observed in complex organisms and could be utilized in spaceflight. Hibernation, estivation, torpor, diapause and its extreme form cryptobiosis, when organism shows no visible signs of life, are good examples of behaviors that may be useful for long duration spaceflight. Changes in metabolic rate of some

organisms has been observed to be reduced by 80%, and nearly 100% in cryptobiotic animals.[12,13] The metabolic suppression is also evident in human-size animals such as the black bear, giant panda, and even to some degree in human.[14,15,16]

Although metabolic suppression is a natural survival response to changes in environmental conditions, it can also be induced in some organisms by altering factors such as temperature or through exposure to selective chemical agents.[17,18,6,7,19,20] With recent advances, it is now possible to deliberately initiate and end dormant states in a variety of animal species that do not naturally hibernate, demonstrating the achievement of metabolic control in a variety of "non-hibernating" species.[21,18,22,6,23,7,24]

Carefully controlled studies that precondition organisms to survive hostile environmental conditions create an opportunity for scientists to investigate metabolic control of organisms for specific purposes related to long duration spaceflight. In the metabolically suppressed state, animals have practically no response to any environmental factors including pathologies and chemical toxicity, which creates an ability to potentially minimize, or even exclude their impacts. This may be particularly useful in long duration spaceflight, and in extraterrestrial planetary environments of altered gravity, radiation, and dusts. By using animal models that can be metabolically controlled, scientists will be able to compare the practical advantages of hibernating organisms to currently used animal models.

In planning for long-duration space missions, the availability of oxygen, water, and food is critical for survival. Intentionally induced metabolic down-regulation may be a useful operational response when the available oxygen and or food supply are limited. One of the most profound hallmarks of metabolic suppression is a quantifiable reduction in food intake during the

stasis. Many non-hibernating animals, which have been used in spaceflight experiments including rodents, do not possess extensive metabolic energy reserves and therefore rely on small fuel stores when food is limited or absent. These animals are in serious danger of death if a source of food is not found quickly. Therefore, intentionally induced metabolic suppression will prolong their survival over extended periods of time in absence of food.

Since humans first starting using animals in space experiments in the 1950s, animal mortality has been a problem in some missions. However, much can be learned from those that survived in ill-fated missions. For example, in the Bion M1 mission, the animal mortality rate was observed to be about 75 %. It was found that all gerbils, most of the 45 mice, and all of the fish did not survive the mission due to equipment failure. In contrast, all geckos and snails onboard Bion-M1 did survive the flight, which demonstrates the tremendous tolerance they have to hostile environmental conditions[4]. Despite the high mortality rate, the Bion-M1 mission has provided important information on the limits of survivability of organisms when exposed to unpredictable failures in life support systems. The mission also demonstrated the importance of careful selection of appropriate animal models to reduce risk of animal mortality.
By incorporating animal models that are capable of metabolic control into long duration space biology experiments, the likelihood of mission success will be greatly enhanced.

Additionally, the mass and power requirement of life support systems can be reduced, making transportation of experimental animals to other planets and deep space destination more feasible. In space biology, differentiating between the biological effects of launch or reentry versus fundamental responses to microgravity has always been a problem. However, this challenge

could be overcome if animals were metabolically pre-conditioned for both launch and the return phases of a mission. By inducing a metabolically controlled state, biological responsiveness to the environmental factors and stress related to launch and reentry could conceivably be eliminated. In a hypothetical experimental design, animals could be activated to a "normal" physiological state during the desired space flight phase allowing researchers to focus completely on spaceflight effects not related to launch or re-entry.

Furthermore, payloads that contain highly-flexible organisms with regard to operations, experiment, and spacecraft loading requirements have significant advantages over payloads with organisms that have limited tolerance and extensive requirements. Placing animals into a reversible hypometabolic stasis prior to launch will also help to solve many pre-flight experiment operational problems related to unpredictable launch delays.

Knowledge gained from the use of animals in suspended animation will provide opportunities to gain insight into the development of mitigation strategies designed to reduce risks associated with long duration human spaceflight. The development of solutions to other biomedical problems where metabolic control is needed may also emerge from this work.

The study of metabolic control in spaceflight will help to address questions formulated in the NASA Fundamental Space Biology (SB) Science Plan and the National Research Council's 2011 Decadal Survey Report question, "How are the basic metabolic rate and metabolism of living systems, including lifespan, affected by spaceflight?"

Examples of microorganisms and animal models with dormancy capability suitable for space research are summarized below. Each of these organisms possess unique metabolic mechanisms and molecular machinery that enable them to survive in what would normally be considered hostile or deadly environments in very unusual ways. These creatures demonstrate features useful for spaceflight metabolic control studies and could provide synthetic biologists information on how to engineer organisms designed to be metabolically controlled during long duration spaceflight.

Microorganisms and small animals as models for planetary colonization: Microbes are the most diverse and abundant type of organism on Earth. They possess billions of years of evolutionary adaptions that have enabled them to survive in the extraordinary wide range of physiochemical environmental conditions that exist above, on, and within the Earth. Microbes also have the ability to suppress metabolism and remain dormant for long periods of time, and is routinely used by a variety of organisms to overcome unfavorable environmental conditions. Approximately 99% of all microbes on Earth are in a dormant state [25,26,27], and only a tiny fraction is metabolically active at any given time. That dormancy and the presence of such large reservoirs of microbial biodiversity have important implications for the stability and functioning of ecosystem services over both short term and geologic time. Consequently, microorganisms that are capable switching to and from the dormant state may outcompete microorganisms with better growth performance in unfavorable environments[28]. These microorganisms affect entire ecosystems on Earth, and may be involved in panspermia. Microbe-colonized rocks could be ejected from Earth during impact processes and fall onto other planets or Moons. Similarly, rocks from other planets, the Moon, and asteroids have landed on the Earth due to impact

processes, so it becomes possible to imagine microbes being transported across the solar system from planet to planet via impact processes. It is reasonable to expect that microbes in a deep metabolic stasis (dormant state) may be ideal candidates to survive such processes. Evidence of the dormancy in microbial species on Earth is a clue that suggests a preference for more favorable environmental conditions that existed in the past. This has implications for panspermia and the search for life on Mars, Europa, Enceladus or other astrobiological targets.

Over last two decades, a great deal of evidence has amassed that demonstrates primitive life forms can survive in space, and even remain viable following direct exposure to the vacuum of space. NASA, ESA, and the Russian Space Agency have conducted experiments with a variety of organisms that were exposed to the hostile environment of outer space, which is permeated by space radiation, experiences extraordinary temperature variations, and is a vacuum. Some of the experimental microbes demonstrated remarkable survivability. Moreover, many of those that did survive were not expected to tolerate these environmental conditions whatsoever.[28] The organisms tested included spores of *B. subtilis*, [29,30,31,32] the lichen *Rhizocarpon geographicum*, *Xanthoria elegans*[33], and adults and eggs of the tardigrades *Richtersius coronifer* and *Milnesium tardigradum*.[34] *B. subtilis* (70%) spores survived 2107 days in space, when shielded against solar ultraviolet radiation[35]. Recently, Russian cosmonauts have found traces of sea plankton and microscopic particles on the exterior surface of the ISS.[36] If such findings can be confirmed, this will provide additional evidence that some organisms can survive in low gravity, extreme temperature conditions and hard cosmic radiation.

Through spaceflight experiments and ground studies, there are accumulative evidences that terrestrial microbes may be able both to survive a trip to Mars aboard a spacecraft and adapt to a new planetary environment under special circumstances.[37,38]

Potentially habitable planetary environments outside of the Earth are exposed to large doses of solar and cosmic radiation, are extremely hot, cold, or experience wide temperature swings, are extremely dry, and are hypersaline. For example, hypersaline environments have been observed on Mars and are expected in the oceans that exist beneath the frozen surfaces of Europa and Enceladus, the moons of Jupiter and Saturn. [39,40,41,42,43] Species that have ability to adapt and survive in similar conditions on Earth will be the most likely candidates for colonization of these planets and moons. Examples include algae, protozoans, fungi cysts, and bacteria that demonstrate cryptobiosis and long periods of dormancy in Antarctic and Arctic ice.[44] Novel ecologies recently discovered in salt crusts of the United Arab Emirates[45] may also contain candidate microbes suitable for metabolic control studies and colonization of extraterrestrial environments.

It is remarkable that several groups of species among the *Tardigrade Ramazzottius*, and the *Ozobranchus jantseanus* are able to survive extremely low temperatures by reversibly suspending their metabolism and going into a state of cryptobiosis.[46] Although these species are not considered extremophilic because they are not normally in ‘extreme’ environmental conditions, their ability to survive when exposed to them is scientifically interesting, and may be useful for space biology, astrobiology, and exobiology research purposes.

It also should be noted that although the surfaces of many extraterrestrial objects are likely sterile and not habitable, the subsurface and interiors of them may be habitable. Life forms that might

exist in the subsurface of extraterrestrial bodies would be protected from harmful conditions on the surface.[47]

Characterization of life forms capable to adapt to adverse conditions such as high and low temperature environments, and be able to reproduce under these conditions, is an important aspect of astrobiology research. Moreover, studies that explore the ability of microbes to survive on materials observed on other worlds like Mars[48] are providing new insight into the ability of life from Earth to exist on present day Mars. However, we must consider the possibility that extraterrestrial environments may be so extreme that they prevent all metabolic processes. Nevertheless, it is important to study microbial survival strategies in hot, cold, hypersaline, and desert environments, to better understand the limits of life and the potential for it to exist elsewhere in the solar system. If life exists on Mars, it likely exists in a dormant, metabolically controlled state within or underneath rocks, or in the subsurface, in ice or salt-rich conditions.

Lichens: Lichens are one of the most appropriate symbiotic systems for seeding and or creating a mini-ecological system on Mars, or on other potentially habitable planets. Lichens are symbiotic associations (holobionts) between fungi (mycobionts) and certain groups of cyanobacteria or unicellular green algae (photobionts). This symbiotic association was essential in the colonization of dry terrestrial habitats.[49] The vast majority of lichens are desiccation-tolerant and can survive in a suspended animation during long periods of drought until water becomes available again, at which time they revive and resume normal metabolism.[49,50] Upon rehydration they recover normal photosynthetic rates within a very short time span. Individual lichens can live for hundreds or even thousands of years.[51] Therefore, lichens may be candidate

life forms for colonization of extreme extraterrestrial environments that are cold, hot, and desert-like.

Lichens also demonstrate a unique symbiotic survival strategy. The fungus creates a niche for its algae, in places where the algae alone would likely not survive. Occasional exposure to water (rain, flooding) let them recharge and store food for the next period of dormancy. Because lichens enable algae to live all over the world in many different climates, they also serve as an important role in the carbon cycle and also produce oxygen. Algae, lichens, and mosses take up approximately 14 billion tons of carbon dioxide and approximately 50 million tons of nitrogen per year from atmosphere and fix it into the Earth's surface.[52]

Scientists are beginning to explore the candidacy of lichens for Mars. Studies exposing lichens to simulated Martian conditions were recently performed to characterize lichen metabolic responsiveness.[53]

Snails: Invertebrates such as snails are convenient test subjects for biological research due to their low weight, cost, and wide range of metabolic activity. Snails are able to survive harsh environments, including cold, hypergravity, hypogravity, and high doses of ionizing radiation (up to 200 Gy).[54] Snails have been flown on several spaceflights (NASA, ESA, Russian, Chinese), for studies of re-adaptation to Earth's gravity following return from space. The edible snail (*Helix pomatia L.*) is able to suppress their metabolism in response to harmful environments such as starvation and hibernate for years.[55] Some snails such as *Oxystyla pulchella* are able to increase their lifespan seven fold by hibernating from three years to twenty-three years.[56] This unique ability gives them significant survival advantages in response to life

threatening conditions when compared to animals with no metabolic flexibility. For example, snails were some of the lone survivors of a recent Bion-M1 experiment (2013) that experienced technical problems, whereas most of the mice and Mongolian gerbils did not survive after 30 days in orbit due to hardware failures. Snails were among the few survivors in that mission.[4]

Taken collectively, we have learned that, snails are excellent candidates for spaceflight metabolic control experiments. Snails can be placed in a stasis, transported to LEO and beyond, where they could be later revived for physiological experimentation.

Leeches: The *Ozobranchus jantseanus* leech found in freshwater turtles in East Asia is able to tolerate extraordinary temperature extremes. In comparison to *Tardigrade Ramazzottius*, and the larvae of the *Chymomyza costata*, which can survive for up to an hour in liquid nitrogen, *Ozobranchus jantseanus* leeches have survived 2.5 years at a temperature of -90°C and in a liquid nitrogen for a full day. The leech was also capable of enduring repeated freeze-thaw cycles in the temperature range 20°C to −100°C and then back to 20°C [57]. If this finding is repeatable by additional studies, it may open an ambitious opportunities for testing planetary "seeding" and adaptation scenarios with this or other species engineered for harsh environments

Arctic caterpillars and ice worms: Arctic woolly bear moth and ice worms are found in Greenland and Canada around the Arctic Circle. Unlike the other caterpillars, *Gynaephora Groenlandica* caterpillars spend about 5% of their lives eating, feeding on the Arctic tundra during the month of June. For the rest of their lives they are dormant. When exposed to freezing temperatures, the caterpillars break down all their mitochondria, alter their metabolism, and start to synthesize glycerol, which acts as an antifreeze to protect the cells from the freezing conditions. In this adaptive state, *Gynaephora* is able to survive in temperatures below −60°C.

As a result, the woolly bear caterpillar has the longest life cycle of any butterfly or moth. It can take up to 14 years to for the insect to complete metamorphosis, from egg to adult moth.[58]

The ice worm, *Mesenchytraeus solifugus*, is among a few metazoan species that not only survives at subzero temperatures but also has the ability to maintain all biological processes at 0°C. Ice worms remain fully functional at freezing temperatures. Compared to their mesophilic counterparts, their response to temperature change is distinctly opposite, namely, ice worms increase production of ATP to off-set, at least in part, the inherent lethargy and death usually associated with cold temperature.[59] Although they both live in similar conditions, the woolly bear caterpillars and the ice worms both employ very different strategies to survive the frost. The ice worms altered their internal structure and metabolism to allow them to function at freezing temperatures, rendering them permanently incapable of living anywhere else.

Turtles: The (*Agrionemys horsfieldii*) Russian turtle has been used in LEO experiments and also been flown around the Moon. Launched on September 15, 1968, the Soviet spacecraft Zond 5 that included onboard two turtles (steppe tortoises) along with others biological payloads made a loop around the Moon and safely returned to earth six days later[60]. The turtles appeared to tolerate spaceflight well, but experienced a 10% weight loss. Tortoises were sent to space again, aboard Soyuz 20 in 1975. That mission kept tortoises in space for 90.5 days, setting a duration record for animals in space.[61,62] In February of 2010, the Iranian Space Agency also utilized turtles in their first biological payload into a sub-orbital flight (Kavoshgar 3).[63]

One of the advantage of turtles as an animal models in space experiments is their ability to combine their low metabolic rate with the ability to further reduce metabolism in response to harsh environmental conditions.[64] This metabolic depression conserves energy which lengthens

the time turtles can survive in hostile conditions and allows them to emerge from these conditions with more resources for growth and reproduction. Turtles in the Zond 5 mission were able to not only survive the round trip to Moon but also survive a ballistic 20 G during reentry to earth[1,65].

Mouse: Rodents have been the most frequently flown mammalian animal model used to study physiological responses to the space environment. Mice are broadly used to study effects of low gravity and space radiation on musculoskeletal system, cardiovascular and immune functions.[66,67,68,69] Although the deleterious effects of bone loss and muscle atrophy appear to be common to most species in microgravity and also in bed rest experiments on humans, recent studies have shown that animals that undertake extended bouts of natural immobility (i.e., dormancy) consistently demonstrate less atrophy than that experienced by the other mammalian models immobilized for considerably less time.[70,71,72,73] Knowledge gained in the use of animals in dormant state in spaceflight could reveal the fundamental mechanisms of adaptation to spaceflight. Such knowledge may provide insights for potential long duration human spaceflight risk mitigation strategies and potential new approaches for solving space biomedical problems. Therefore, mice, with intentionally-induced metabolic suppression may be useful subjects for developing experimental models for testing the efficacy of metabolic control and its effect on bone loss and muscle atrophy during spaceflight.[6,7,74] Long duration studies will allow scientists to explore the possibility of manipulating metabolic mechanisms to reduce the negative effects of multiple space environmental factors on animals and humans and preserve their life during a long duration space mission.

Dormouse: The edible dormouse (*Glis glis*) is a small arboreal rodent that has an extremely long life span of up to 9 years, and is able to survive cold temperatures and food restriction during a hibernation period of up to eight months. These hibernators have the ability to reduce their metabolic rate to a fraction of their basal energy requirements and lower their body temperature nearly to external ambient temperatures. *Glis glis* genome has recently been sequenced providing researchers opportunities to identify key underlying molecular pathways of its protective metabolic control adaptations.[75] Taken collectively, the dormouse is an attractive animal model for spaceflight experiments aboard the ISS or free-flying biosatelites.

Pocket mouse: *Perognathus longimembris* is another facultative homeotherm with the ability to dramatically suppress its metabolic rate in response to environmental stress[76]. This species has the ability to produce water metabolically from its food, and thus does not require a supply of drinking water. Due to this capability as well as its small size, pocket mice were selected for the Apollo 17 moon orbital mission, which demonstrated high rate of survival in the BIOCOR experiments[77,78]. Later, in July 1973, pocket mice were also used as model organisms in the Skylab 3 mission to study the stability of circadian rhythms during orbital spaceflight[78].

Squirrels: Arctic squirrels *(Spermophilus parryii)* are able to reduce their core body temperature to -2.9˚C in the wild, while keeping their brain temperature just above 0˚C.[79] The physiological changes in the brain of hibernating squirrels are most likely caused by the effects of low temperatures resulting in significant reduction of their neural activity. Arctic squirrels lose up to 60% of their neural synapses during hibernation. However, these connections are later rewired when the animal emerges from hybernation.[80] The animal experiences a massive resprouting of

neural networks, enabling their learning and memory performance to improve. This suggests that the loss of synapses during hibernation could actually be beneficial for learning about the new features of their environment that may have changed over the winter months.[81]

Although they are classically used in hibernation research, Arctic squirrels could be an appropriate model for space biology, to address questions that investigate cognitive integrity after prolonged exposure to spaceflight conditions. For example, scientists have discovered that the protein RBM3 helps synapses to rebuild in animals awaking from hibernation, which restores normal brain activity. Humans also have the protein, but in some clinical examples it appears not to function, such as in populations of people with Alzheimer's disease. Researchers are hopeful that a drug that mimics or increases the effectiveness of RBM3 could help restore lost brain functions for people with dementia. Interestingly, this protein may also be involved in hypothermia. Researchers have found that metabolic suppression due to hypothermia can protect the brain from some forms of damage, and this phenomenon is being investigated for the development of treatment methods for strokes and other forms of acute brain injury. A similar protective mechanism now also being studied in neurodegenerative disease.[82] These and other lines of research may help to mitigate or even solve cognitive problems that astronauts may experience during long term exposure to spaceflight radiation and microgravity.

The role of the synaptic activity and cognitive ability is an area of important study for the NASA Human Research Program, regarding potential changes in cognitive abilities of astronauts during exposure to space radiation and microgravity during future long duration spaceflight. Understanding the neurocognitive and neuropsychological parameters influencing astronauts during space flight is of high relevance with regard to future human missions to Mars.

It has been shown that exposure to the dangerous radiation fields in space impair the cognitive abilities of rodents, suggesting that astronauts who spend extended time in space may suffer similar consequences.[83] Other environmental characteristics specific to space missions may affect neurocognitive performance presenting a serious risk to mission success. Understanding the neurobiological processes underlying cognitive impairment caused by spaceflight in animal models with metabolic flexibility may allow researchers to identify and develop optimal behavioral and pharmacological countermeasures for astronauts.

Small primates: The fat-tailed dwarf lemur (*Cheirogaleus medius*) is closest genetic relative to humans and is the first primate in whom hibernation was observed. The adult lemur mass is ~160 grams and its maximum lifespan in captivity is nearly 30 years.[84] They are able to hibernate for 7 months at relatively high environmental temperatures after storing fat in its tail, which provides the needed energy source during periods of dormancy. During hibernation, the animal's metabolic activity decreases to about 2 percent of its normal active metabolism, and its body temperature drops to match the ambient temperature. As a primate, the fat-tailed dwarf lemur is probably the best animal model that can be used to explore the potential for hibernation in humans.

During hibernation that is accompanied by hypothermia, the mammalian brain does not generate the electrical activity that observed during conventional sleep. That led researchers to hypothesize that the need for sleep may explain why mammals periodically wake up from hibernation. In contrast to most hibernating animals (which rarely or never enter REM sleep during hibernation), the brain of hibernating dwarf lemurs is unique because it enters into REM-sleep for unusually long periods.[85] This may be due to the fact that unlike many smaller hibernators, dwarf lemurs body temperatures during hibernation do not drop significantly. As a

result, these unique neurological features make the organism an attractive model for exploring sleep and temperature-metabolism relationships. Because of its genetic similarity to humans, the underlying regulatory mechanisms for genes in fat-tailed dwarf lemur are very important, and suggests that investigations that study practical hibernation or torpor in humans may be warranted.

In summary, spaceflight experiments with animals with ‘normal metabolism’ have many restrictions and limitations, thus studies of animals, such as those we have discussed, in a stasis may be excellent candidates for long duration studies, due to reduced life support system requirements and reduced animal activity. Metabolically controlled animals may be well suited for unmanned spacecraft as well.

Further development of methods to achieve hypometabolic stasis or other modes of dormancy: Development of research programs that define the optimum methods for induction of the dormant state and achieving metabolic control in key experimental animal systems is currently needed. Such an effort will support the development of validated protocols that can be used for transportation of metabolically controlled model organisms. It is imperative that metabolic control strategies be tested both on the ground and in space. This work will create the foundations for the development of operational scenarios useful for long duration lunar, Mars, and deep space exploration.

Incorporation of the metabolic control strategy in animal spaceflight architecture: In planning for long-duration space missions, the availability of oxygen, water, and food is critical for survival. Intentionally induced metabolic down-regulation may be a useful life-support adjunct when an oxygen or food supply is limited. One of the most profound hallmarks of metabolic suppression is the dramatic reduction in food intake during the stasis. Many non-hibernating animals, which have been used in spaceflight experiments including rodents, do not possess extensive metabolic energy reserves and therefore rely on small fat stores when food is available, limited, or absent. These animals are in serious danger of death if a source of food is not found quickly. Therefore we predict that intentionally induced metabolic suppression will prolong survival over extended periods of time in absence of food.

Development of self-contained animal transportation systems: Over the next 5-10 years, a new animal research program oriented towards space research should be developed, including the required supporting technologies to accommodate metabolic control-based animal studies in LEO, on the Moon, on Mars and in deep space. The program should align with the National Research Council (NRC) Decadal Survey on NASA Life and Physical Sciences, and be executable by either robotic and or human-tended missions that also support fundamental space biology research and elements of the NASA Human Research Program. The program should also be designed to have a flexible path or phased approach, allowing discovery to guide follow-on technology and operational scenario development relevant for future long duration human space exploration.

A robust program of metabolic control research and technology innovation will help to prepare for eventual human travel and presence beyond low earth orbit, on near-earth-objects, and on the surface of, or in orbit around, the Moon, Mars, and beyond.

To date, NASA has already demonstrated some interest in the development of such technology, providing funding to SpaceWork Inc. through the NASA Innovative Advanced Concepts (NIAC) research program, toward scenario drafting and assessment of engineering concept for this technology in application to human mission to Mars.[86]

Animal studies incorporating metabolic control technology would ideally address the following objectives: a) demonstrate animal presence in the environments of deep space as well as on or near the Moon and Mars; b) demonstrate protective and useful effects of metabolic suppression against harmful effects of space radiation and altered gravity, and transit- and destination-specific environmental conditions at the sub-cellular and whole organism level; c) provide preliminary data and assessments for robotic precursor missions incorporating Metabolic Control technology in future objectives for space colonization.

Thorough evaluation of the effects of the space environment on model organisms and mammalian systems, and our ability to perform metabolic control, will require a combination of both *in situ* evaluation and sample return. Small, light-weight physiological monitoring systems will need to be developed to provide critical real-time information as biological experiments unfold. If possible, select biological specimens should also be returned to Earth to allow for detailed studies such as 'omics' analyses that are not currently possible in spaceflight.

The benefit of implementing metabolic suppression into operational scenarios may also enable the establishment of complex ecologies on and within other planetary surfaces. For example, by using dormant, “metabolically suspended” plants and animals, one can envision the actual possibility of future colonization of the nearest planets.[87] Hypometabolic stasis is suitable for such suspended ecosystems due to the ease of transport in space for extended periods without special care as compared with an active ecosystem. Also, storage of seeds and metabolically suspended animals would provide a reserve in case the active part of an ecosystem is negatively affected by unpredictable space hazards.

At this time, there is great public interest in the efforts of both NASA and private companies to send humans to Mars in the near future. However, this ambitious plan is not yet supported by results from animal models. It is simply unknown if humans will survive a prolonged voyage to and from Mars. Moreover, a report on the ethics of long-term spaceflight prepared for NASA by panel of scientists at the National Academy of Sciences in April 2014 stated that such travel potentially includes a "wide range of risks that are poorly characterized, uncertain, and perhaps unforeseeable." Therefore, it is imperative that the feasibility of a humans to Mars mission be first tested with an animal “pathfinder” mission, one perhaps similar to the Fobos-grunt mission.[88,89]

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